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The use of an interferon-gamma release assay to screen for pediatric latent tuberculosis infection in the eastern region of the Emirate of Abu Dhabi

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SUMMARY

Objectives: Intense migration to the United Arab Emirates from tuberculosis (TB) high-endemic areas presents a particular risk to the population. Screening for latent tuberculosis infection (LTBI) usually involves risk assessment, the tuberculin skin test (TST), and interferon-gamma release assay (IGRA). This study investigated the use of an IGRA to screen for LTBI and compared its performance with a risk assessment questionnaire.

Methods: This prospective cross-sectional study was conducted at seven Ambulatory Healthcare Services facilities in Abu Dhabi. Participants (88% Emiratis) were pediatric patients presenting for routine care. The QuantiFERON-TB Gold In-Tube test was performed and the parents completed a questionnaire assessing TB risk factors.

Results: Six-hundred and ninety-nine subjects (median age 8.7 years, interquartile range 9.2 years) were enrolled; 669 (96%) agreed to testing. Four patients had a positive IGRA; one had previously been treated for TB, resulting in three patients with LTBI. The estimated LTBI prevalence was 0.45% (95% confidence interval 0.09–1.3). A household contact from a TB high-endemic area was reported in 44%, travel to a TB high-endemic area in 10%, and contact with someone with a chronic cough in 7%, a TB case in 3%, a TST-positive case in 2%, and an IGRA-positive case in 2%. Fifty percent of participants had at least one risk factor. The risk assessment did not predict a positive IGRA.

Conclusions: The questionnaire yielded a risk of TB exposure of 50%, however the LTBI prevalence, as defined by the IGRA, was low (0.45%).

1. Introduction

The United Arab Emirates (UAE) is considered a tuberculosis (TB) low-endemic country, with a TB disease prevalence of 2.4 per 100 000 population.[1] Increased travel from TB high-endemic areas, however, presents a particular risk to the population. In a recent study, the prevalence of pulmonary TB in the visa screening program was 39 per 100 000.[2] The infection was more common in nursery workers, housemaids, and private drivers.[2] The prevalence of latent tuberculosis infection (LTBI; positive QuantiFERON-TB assay) in Emirati medical students, on the other hand, was found to be 8%.[3] Thus, the UAE population (especially children) is at high risk of TB exposure, perhaps stressing the need for universal screening using the tuberculin skin test (TST) or an interferon-gamma release assay (IGRA).[4,5]

Approximately a third of individuals exposed to TB are expected to develop LTBI, and each case has a life-long risk of progressing to TB.[6] Treatment with isoniazid reduces this risk by at least 75%.[7] Thus, untreated LTBI constitutes a significant epidemic reservoir. The propensity to develop TB disease is much higher in young children.[8] Therefore, effective pediatric screening is vital.

The TST is the standard method for determining TB exposure (immune response to *Mycobacterium tuberculosis*). In the UAE, the bacillus Calmette–Guérin (BCG) vaccine is administered to all

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neonates. In one study, the estimated prevalence of a false-positive TST (≥10 mm induration attributable to BCG) was 8.5% within the first 10 years of BCG administration and 1% thereafter.[9] The prevalence of a false-positive TST from non-tuberculous mycobacteria is also significant, ranging from 0.1% to 2.3%.[9] These false-positive TST reactions discourage physicians and families from accepting treatment and also result in the treatment of some children who are not infected.

The IGRA is based on the release of interferon-gamma (IFN-γ) by T-lymphocytes in response to the *M. tuberculosis* antigens ESAT-6, CFP-10, and TB7.7(p4), which are lacking in BCG vaccines and most non-tuberculous mycobacteria. The higher specificity of the IGRA may permit its use in identifying patients likely to benefit from TB therapy.[10,11] This assay, however, has significant limitations, including a within-subject inter-laboratory variability of about 6%.[12–14]

This study investigated the use of an IGRA to screen for LTBI in pediatric patients from the eastern region of Abu Dhabi. The use of a risk assessment questionnaire to guide LTBI screening was also investigated.

2. Methods

2.1. Study population

This prospective cross-sectional study was conducted at seven Ambulatory Healthcare Services (AHS) centers in the eastern region of the Emirate of Abu Dhabi (UAE). These facilities serve mostly urban populations. From April 29 to October 31, 2013, patients aged 1–19 years presenting for routine care were recruited randomly. The study was approved by the Institutional Review Board of the College of Medicine and Health Sciences (CMHS), UAE University (Protocol #13/14). Written informed consent was obtained for each participant. For the young and those unable to read, consent was obtained from the parents.

2.2. Data collection

Demographic data were collected. The parents completed a modified risk assessment questionnaire (developed by the Pediatric Tuberculosis Collaborative Group and American Thoracic Society[4]) (Table S1, **Supplementary Material**). The questionnaire was administered face-to-face by a trained nurse. The questions were given in the primary language of the families, via an interpreter when necessary.

The QuantiFERON-TB Gold In-Tube test (Cellestis Limited, Carnegie, Victoria, Australia) was performed on enrolled participants who consented to the test. The assay was performed as per the manufacturer's instructions. The QuantiFERON-TB Gold collection tubes were validated for volumes of 0.8–1.2 ml (black marks on the side of tubes indicate 1-ml fill volume). Briefly, approximately 1 ml of blood was collected by venipuncture directly into each of the following tubes: (1) heparin (nil), (2) heparin + dextrose + phytohemagglutinin A (mitogen), and (3) heparin + dextrose + TB antigens (ESAT-6, CFP-10, and TB7.7(p4)). Tubes were shaken vigorously (10 times) to ensure the entire inner surface had been coated with the blood. The samples were transferred to an incubator set at 37 °C within 8 h of collection and incubated for 16–24 h. The tubes were then centrifuged at 25 °C and approximately 3000 *g* for 15 min. The plasma was stored at 2 °C for <4 weeks. Plasma IFN-γ was determined by ELISA using the reagents supplied. The results were interpreted as per the manufacturer's instructions (Table S2, **Supplementary Material**).

Children with a negative QuantiFERON-TB Gold In-Tube test result were not evaluated further. Children with a positive test result underwent a clinical assessment, the TST (using the Mantoux technique), and a chest X-ray. For the purpose of this study, LTBI was defined as a positive IGRA in a child without prior TB disease and with a normal clinical assessment and chest X-ray. These children were offered standard LTBI treatment.

2.3. Statistical analysis

With a reported prevalence of positive TST of 10% in UAE children[15] and a similar prevalence of positive IGRA in healthy medical students of the College of Medicine and Health Sciences (UAE University),[3] a sample size of 553 subjects was estimated to give study precision of 2.5% with 95% confidence levels (OpenEpi, version 2; open source calculator: http://www.openepi.com). With an estimated 30% of participants expected to have either incomplete data or to refuse blood sampling, the required sample size was increased to 699. The primary outcome was estimating the prevalence of LTBI. The secondary outcomes were assessing TB risk factors in the questionnaire and correlating them with the IGRA results. The dependent variable or outcome was IGRA results. The prevalence of LTBI was calculated with 95% confidence intervals (95% CI). The independent variables included ethnicity, age, gender, and risk of TB exposure. Univariate analysis of the risk factors associated with a positive IGRA included non-parametric tests, such as Kruskal–Wallis one-way analysis of variance for independent data (as data did not follow a normal distribution). Proportions were compared with the Chi-square test or Fisher's exact test (if numbers were small). The analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, IL, USA) and the statistical significance was defined by a two-tailed p-value of <0.05.

3. Results

Six-hundred and ninety-nine participants were enrolled in the study and completed the questionnaire. Their median age was 8.7 years (interquartile range 9.2 years). The age distribution was: <1 year, 5 (0.7%); 1–2 years, 41 (5.9%); 2–5 years, 143 (20.5%); 5–10 years, 198 (28.3%); 10–15 years, 168 (24.0%); and >15 years, 144 (20.6%). There were 322 males (46%) and 617 (88%) Emiratis (Table 1).

Only 669 (96%) participants agreed to blood sampling. The remaining 30 (4%) did not differ significantly in gender, nationality, or any variable in the questionnaire ($p > 0.05$). Those who declined blood sampling were younger (<10 years of age, 6.2% vs. 2.5%, $p = 0.03$) and less likely to have a sibling participating in the study (1.7% vs. 6.4%, $p = 0.01$).

Although all 699 questionnaires were completed, 2–8% of the answers were incomplete. BCG administration was reported in 79% (477/601), prior positive TST in 0.47% (3/639), and prior positive

Table 1
LTBI in the studied pediatric population[a]

	Total participants	Participants with IGRA	LTBI prevalence[b]
Overall	699 (100%)	669 (96%)	3 (0.45%)
Gender			
Male	322 (46%)	305 (43%)	1 (0.33%)
Female	377 (54%)	364 (52%)	2 (0.55%)
Nationality			
Emirati	617 (88%)	592 (91%)	3 (0.51%)
Non-Emirati	65 (9%)	60 (91%)	0 (0%)
Unknown	17 (2%)	17 (0%)	0 (0%)

LTBI, latent tuberculosis infection; IGRA, interferon-gamma release assay.
[a] Results are expressed as *n* (%).
[b] LTBI prevalence was set as the number of LTBI cases/participants with IGRA results. LTBI was defined as a positive IGRA in a child without prior TB disease and with normal clinical assessment and chest X-ray. Four patients had a positive IGRA; one of these patients had previously been treated for TB disease.

Table 2
Patients with a positive IGRA

	LTBI			Previous TB
	Patient 1	Patient 2	Patient 3	Patient 4
Gender	Male	Female	Female	Male
Age, years	5	18	18	14
Emirati	+	+	+	+
Received BCG at birth	+	+	+	+
Had previous positive TST	–	–	–	+
Had previous positive IGRA	–	–	–	–
Previously treated for TB	–	–	–	+
IGRA reading	0.67	0.77	0.77	2.06
Risk factors				
Household contact from TB endemic area	–	–	–	+
Travel to TB endemic area	–	–	–	NA
Contact with TB case	–	–	–	–
Contact with TST-positive case	–	–	–	–
Contact with IGRA-positive case	–	+	–	–
Contact with chronic cough	–	–	–	–
Total number of risk factors	0	1	0	1

LTBI, latent tuberculosis infection; TB, tuberculosis; BCG, bacillus Calmette–Guérin; TST, tuberculin skin test; IGRA, interferon-gamma release assay; NA, not available.

IGRA in 0.15% (1/677). Siblings participating in this study occurred in 41% (254/617).

Of the 669 tested, 660 participants had a negative IGRA (99%), four (0.6%) had a positive IGRA, two (0.3%) had an indeterminate result (a 3.1-year-old male and a 7.2-year-old female), and one (0.15%) participant had ineligible collection (missing mitogen tube sample). Details of the four patients with a positive IGRA are given in Table 2. One patient had previously been treated for TB disease. Thus, only three LTBI cases were identified. The overall prevalence of LTBI was 0.45% (95% CI 0.09–1.3) (Table 1). The prevalence was 0.28% (95% CI 0.07–1.5) in children <10 years of age and 0.66% (95% CI 0.08–2.3) in older children ($p = 0.4$). None of the patients reported an immune deficiency illness. The IGRA results had no significant associations with any of the risk factors elicited, neither by the questionnaire nor by the number of risk factors per child ($p > 0.05$).

According to the questionnaire, 351 (50%) participants had no risk factors (Table 3); of these participants, 337 were tested and two (0.6%) had LTBI. Thus, these two patients would have been missed by the questionnaire alone.

At least one risk factor was present in 348 (50%) participants (Table 3), of whom 330 were tested by IGRA: only one participant (0.3%) had LTBI while 329 (99.7%) had negative results. Thus, over 99% of participants identified with at least one risk factor would have been investigated unnecessarily.

Table 3
Risk-assessment results[a]

Risk factors	All respondents ($N = 699$)
Household contact from TB endemic area	303/694(44%)
Travel to TB endemic area	68/684 (10%)
Contact with TB case	19/697 (3%)
Contact with TST-positive case	16/698 (2%)
Contact with IGRA-positive case	17/696 (2%)
Contact with chronic cough	51/697 (7%)
Number of risk factors per participant	
0	351 (50%)
1	265 (38%)
2	61 (9%)
3	7 (1%)
4	9 (1%)
5	6 (1%)
6	0 (0%)
At least 1 risk factor	348 (50%)

TB, tuberculosis; TST, tuberculin skin test; IGRA, interferon-gamma release assay.
[a] Because of incomplete entries, the results are expressed as *n*/total respondents (%) unless stated otherwise.

The distribution of risk factors and LTBI patients by center are shown in the **Supplementary Material** (Table S3). Interestingly, the three LTBI patients were from Hilli center, and only 20% of the participants in this center reported ≥1 TB risk factor. In Maqqam center, 94% of the participants reported ≥1 TB risk factor, but no LTBI was found (Table S3, **Supplementary Material**).

4. Discussion

In this study, an IGRA was used to estimate the prevalence of pediatric LTBI in the eastern region of Abu Dhabi. The overall prevalence of LTBI was 0.45% (95% CI 0.09–1.3). As the IGRA is known to have lower sensitivity in young children,[16] the reported prevalence of LTBI is probably an underestimate.

Participants over 10 years of age had an LTBI prevalence of 0.66% (95% CI 0.08–2.3). This value is lower than the 8% found in a recent study involving medical students in UAE,[3] suggesting TB exposure increases with age.

The use of questionnaires to assess the risk of TB exposure is not implemented in the UAE. In a diverse population from the Northern California Region, children 0–18 years of age who received routine TST as part of universal screening were assessed for TB risk factors. The prevalence of a positive TST was 1.0%. Risk factors associated with a positive TST included a history of receiving the BCG vaccine (odds ratio 2.31, 95% CI 1.70–3.13) and a household member with a positive TST or TB disease (odds ratio 1.53, 95% CI 1.14–2.04).[4] The authors suggested a five-item risk assessment questionnaire that accurately identified risk factors associated with LTBI in US children; the items were: child received BCG vaccine, household member with a history of TB, child born outside the USA, child lived outside the USA, and household member born outside the USA.[4]

This study showed that for each 169 participants with no risk factor on the questionnaire, one (0.6%) would have LTBI. In addition, for each 330 participants with at least one risk factor on the questionnaire, one (0.3%) would have LTBI. The performance of the questionnaire in our community would, therefore, lead to a few missed LTBI cases and numerous unnecessary investigations. However, regional variations may have an impact on these estimations (Table S3, **Supplementary Material**). Therefore, implementing screening policies requires careful assessment of the TB prevalence in the region.

This study had limitations. The recruitment of participants from outpatient clinics (instead of schoolchildren, for example) invoked a selection bias. The responses to the risk assessment questionnaire were not verified by a single observer (e.g., unsure of prior BCG or

TST, incomplete data entry, recall bias, etc.). Eighty-eight percent of the participants were Emiratis, and LTBI occurred exclusively among them. This selection was due to the health insurance for Emiratis, which covered AHS visits where the IGRA was offered. Most expatriates attend other clinics. The performances of the questionnaire and of the IGRA were not compared to the TST, as BCG vaccination is offered to all children born in the UAE.

Two important findings were identified in this study. First, using the IGRA, the estimated prevalence of LTBI in the studied pediatric population was 0.45% (95% CI 0.09–1.3). Second, 44% of the participants had a household contact from a TB endemic area, 10% had traveled to a TB endemic area, and 3% had had contact with a TB case. Thus, based on risk factor assessment, half of the UAE pediatric patients are eligible for TB screening, although most (99.7%) will not have LTBI.

The relatively low prevalence of LTBI (0.45%) perhaps reflects protection offered by residence visa screening, BCG vaccination at birth, or other undetermined variables. Further studies are required before implementing the IGRA as a screening tool in the UAE pediatric population, including investigations in other regions of the country (urban and rural) and studying the cost-effectiveness of the IGRA.

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Conflict of interest: The authors have no conflicts of interest to disclose.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijid.2013.12.020.

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